abcam

Product datasheet

Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade ab9051

★★★★★ 18 Abreviews 129 References 7 Images

Overview

Product name Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade

Description Rabbit polyclonal to Histone H4 (mono methyl K20) - ChIP Grade

Host species Rabbit

Tested applications Suitable for: IHC-P, IHC-Fr, Flow Cyt (Intra), WB, ICC/IF, ChIP, ChIP-sequencing, IHC -

Wholemount

Species reactivity Reacts with: Mouse, Cow, Human, Caenorhabditis elegans, Drosophila melanogaster,

Schizosaccharomyces pombe

Predicted to work with: Mammals

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Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

General notes Interphase: The cells within the culture show a considerable variability in the intensity of staining

with the antibody. The relationship between trimethylation levels and cell cycle have not yet been determined but may be a contributor to the amount of methylation detected in each cell. Heterochromatic regions of the interphase nucleus are the primary sites of trimethylation

observed by indirect immunofluroescence. Mitosis: Discrete chromosomal regions are labelled intensely, with lower level fluorescence throughout the remainder of the chromosome arms Data from IF performed by Kirk McManus in the lab of Michael Hendzel as part of the antibody

characterisation at cellnucleus.com

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

1

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity Immunogen affinity purified

Primary antibody notes Interphase: The cells within the culture show a considerable variability in the intensity of staining

with the antibody. The relationship between trimethylation levels and cell cycle have not yet been determined but may be a contributor to the amount of methylation detected in each cell. Heterochromatic regions of the interphase nucleus are the primary sites of trimethylation observed by indirect immunofluroescence. Mitosis: Discrete chromosomal regions are labelled intensely, with lower level fluorescence throughout the remainder of the chromosome arms Data from IF performed by Kirk McManus in the lab of Michael Hendzel as part of the antibody

characterisation at cellnucleus.com

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab9051 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P	★★★★★ (2)	Use a concentration of 0.05 - $0.2~\mu g/ml$. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IHC-Fr	**** <u>(1)</u>	Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab171870</u> - Rabbit polyclonal lgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (7)	1/1000.
ICC/IF	★★★★ <u>(5)</u>	Use at an assay dependent concentration. Cautionary note: At low dilutions, the antibody may cross-react with microtubules. This can be minimized by increasing the dilution of the antibody when used in immunofluroescence.
ChIP	★★★★★ (2)	Use 2-25 µg for 25 µg of chromatin.
ChIP-sequencing		Use at an assay dependent concentration. PubMed: 20200201
IHC - Wholemount	*** <u>(1)</u>	Use at an assay dependent concentration.

Target

Function

Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling.

Sequence similarities

Post-translational modifications

Belongs to the histone H4 family.

Acetylation at Lys-6 (H4K5ac), Lys-9 (H4K8ac), Lys-13 (H4K12ac) and Lys-17 (H4K16ac) occurs in coding regions of the genome but not in heterochromatin.

Citrullination at Arg-4 (H4R3ci) by PADI4 impairs methylation.

Monomethylation and asymmetric dimethylation at Arg-4 (H4R3me1 and H4R3me2a, respectively) by PRMT1 favors acetylation at Lys-9 (H4K8ac) and Lys-13 (H4K12ac).

Demethylation is performed by JMJD6. Symmetric dimethylation on Arg-4 (H4R3me2s) by the PRDM1/PRMT5 complex may play a crucial role in the germ-cell lineage.

Monomethylated, dimethylated or trimethylated at Lys-21 (H4K20me1, H4K20me2, H4K20me3). Monomethylation is performed by SET8. Trimethylation is performed by SUV420H1 and SUV420H2 and induces gene silencing.

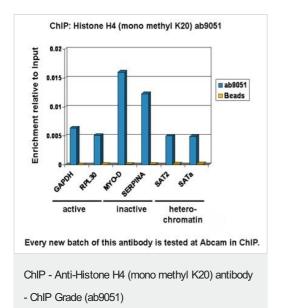
Ubiquitinated by the CUL4-DDB-RBX1 complex in response to ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate DNA accessibility to repair proteins. Monoubiquitinated at Lys-92 of histone H4 (H4K91ub1) in response to DNA damage. The exact role of H4K91ub1 in DNA damage response is still unclear but it may function as a licensing signal for additional histone H4 post-translational modifications such as H4 Lys-21 methylation (H4K20me).

Sumoylated, which is associated with transcriptional repression.

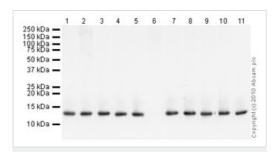
Cellular localization

Nucleus. Chromosome.

Images



Chromatin was prepared from U2OS cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 μg of chromatin, 2 μg of ab9051 (blue), and 20 μl of protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach). Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade (ab9051) **All lanes :** Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade (ab9051) at 1 μg/ml

Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate (ab121)

Lane 2 : Calf Thymus Histone Preparation Nuclear Lysate (<u>ab121</u>) with Human Histone H4 peptide (<u>ab14963</u>) at 0.5 μg/ml

Lane 3 : Calf Thymus Histone Preparation Nuclear Lysate (<u>ab121</u>) with Human Histone H3 (mono methyl K4) peptide (<u>ab1340</u>) at 0.5 µg/ml

Lane 4 : Calf Thymus Histone Preparation Nuclear Lysate (<u>ab121</u>) with Human Histone H3 (di methyl K4) peptide (<u>ab7768</u>) at 0.5 µg/ml

Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate (<u>ab121</u>) with Human Histone H3 (tri methyl K4) peptide (<u>ab1342</u>) at 0.5 µg/ml

Lane 6 : Calf Thymus Histone Preparation Nuclear Lysate (<u>ab121</u>) with Human Histone H4 (mono methyl K20) peptide (<u>ab17043</u>) at $0.5~\mu\text{g/ml}$

Lane 7 : Calf Thymus Histone Preparation Nuclear Lysate (<u>ab121</u>) with Histone H4 peptide (17-24) - di methyl K20 at 0.5 μg/ml **Lane 8 :** Calf Thymus Histone Preparation Nuclear Lysate (<u>ab121</u>)

with Human Histone H4 (tri methyl K20) peptide (<u>ab17567</u>) at 0.5 µg/ml

Lane 9 : Calf Thymus Histone Preparation Nuclear Lysate (<u>ab121</u>) with Human Histone H3 (mono methyl K9) peptide (<u>ab1771</u>) at 0.5 µg/ml

Lane 10 : Calf Thymus Histone Preparation Nuclear Lysate ($\underline{ab121}$) with Human Histone H3 (di methyl K9) peptide ($\underline{ab1772}$) at 0.5 μ g/ml

Lane 11 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K9) peptide (ab1773) at 0.5 μ g/ml

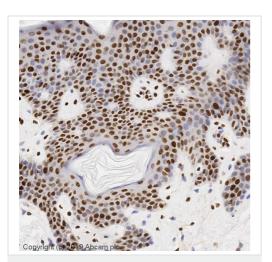
Lysates/proteins at 0.5 µg per lane.

Secondary

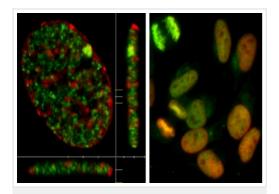
 $\begin{tabular}{ll} \textbf{All lanes:} Goat polyclonal to Rabbit lgG - H\&L - Pre-Adsorbed (HRP) at 1/3000 dilution \end{tabular}$

Performed under reducing conditions.

Observed band size: 13 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade (ab9051)



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade (ab9051)

This image is courtesy of Kirk McManus in the lab of Michael Hendzel, Univeristy of Alberta

Exposure time: 10 seconds

IHC image of Histone H4 (mono methyl K20) staining in Human normal skin formalin fixed paraffin embedded tissue section*, performed on a Leica BondTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab9051, 0.05µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

Rabbit polyclonal to Histone H4 mono methyl K20 (1/250).

HeLa cells cultured on glass coverslips were fixed with 4% paraformaldehyde and then stained with ab9051 (green). Total chromatin was visualized using DAPI staining (red).

This image is part of the antibody characterisation at www.cellnucleus.com

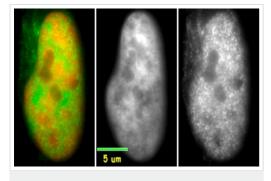


Western blot - Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade (ab9051)

Rabbit polyclonal to Histone H4 mono methyl K20 at 1/1000 on histone prep (Sigma).

Peptide competition (shown above lanes) at 1ug/ml.

This peptide blocking study shows that ab9051 is specific for Histone H4 (mono methyl K20). The activity of the antibody is specifically blocked by the addition of the H4 mono methyl peptide (ab17043).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade (ab9051)

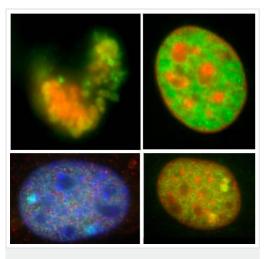
This image is courtesy of Kirk McManus in the lab of Michael Hendzel, Univeristy of Alberta

Rabbit polyclonal to Histone H4 mono methyl K20 (1/250).

HeLa cells cultured on glass coverslips were fixed with 4% paraformaldehyde and then stained with ab9051 (green). Total chromatin was visualized using DAPI staining (red).

Left: merge; center: DAPI; right: Mono K20

This image is part of the antibody characterisation at www.cellnucleus.com



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade (ab9051)

This image is courtesy of Kirk McManus in the lab of Mchael Hendzel, Univeristy of Alberta

Rabbit polyclonal to Histone H4 mono methyl K20 (1/250).

Mouse 10T1 cells cultured on glass coverslips were fixed with 4% paraformaldehyde and then stained with ab9051.

Top left: metaphase cell. Mono methyl K20 (green); DAPI (red)
Top right: interphase cell. Mono methyl K20 (green); DAPI (red)
Lower left: interphase cell. Mono methyl K20 (green); DAPI (blue),
TBP (red)

Lower right: interphase cell. Mono methyl K20 (green); DAPI (red)

This image is part of the antibody characterisation at www.cellnucleus.com

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